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(54) Title: DRUG DELIVERY SYSTEM

(57) Abstract

A drug delivery composition comprising microspheres containing at least one chemotherapeutic agent and at least one chemosensitizer wherein the microspheres have a biodegradable polymer matrix with functional groups which associate with the chemotherapeutic agent and chemosensitizer.

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Title: DRUG DELIVERY SYSTEM

FIELD OF THE INVENTION

The present invention relates to a drug delivery system, and more particularly to a system which enhances delivery of two or more drugs which act together.

BACKGROUND OF THE INVENTION

Chemotherapy with anti-cancer drugs is usually limited by its chronic cardiotoxicity, immunosuppressive activity, and necrotic reaction at the injection site. Although regional therapy (e.g. intraarterial infusion of anticancer drug into the arterial leading to a tumor) has been used to reduce the systemic toxicities, high local toxicity is still a problem. There is a need to improve current chemotherapy to reduce toxicity of the chemotherapeutic agents.

Attempts have been made to reduce the toxicity of chemotherapeutic agents by incorporating the agents into a delivery vehicle to target to a particular site. Typically, drug delivery vehicles are formed as aqueous carriers, gels, polymeric material inserts, or particulates incorporating the agent. Once the drug delivery vehicle is placed at a desired site, the agent is released over a prolonged length of time. The time release of the agent will depend on factors such as the release mechanism of the agent from the drug delivery vehicle (e.g. erosion or diffusion), the amount of agent in the drug delivery vehicle, the solubility of the drug in the surrounding physiological medium, and in the case of particulate delivery vehicles, the particle size or size distribution of the vehicle.

A drug delivery system is desired that enhances delivery of the chemotherapeutic agent to the target organ and prevents loss of the agent in the efferent venous drainage from the organ. Microspheres and microcapsules, (collectively referred to as "microparticles") have been described for delivery of active agents to target organs. Due to their size, they are trapped in the microvasculature of tissues when administered via the regional arterial, where they release their payload. This process is referred to as "chemoembolization". Chemoembolization has been reported to be useful in the treatment of inoperable liver tumors, pain control of bone lesions, as a preoperative adjuvant for locally invasive tumors, and in the treatment of solid tumors in liver, kidney, breast, lung, and head and neck (T. Kato, Microspheres and Regional Cancer Therapy, CRC Press, Boca Raton (1994)). Animal and human pharmacokinetics studies have shown enhanced drug exposure to tumors and diminished systemic toxicity by chemoembolization as compared with regional organ perfusion of the free drugs.

In one study, microspheres prepared using ion-exchange principles were reported to exhibit high doxorubicin loading capacity (>30%), whereas those using chemical cross-linkage and physical entrapment approaches, display drug levels less than 15% (N. Wilmott and J. Daly, Microspheres and Regional Cancer Therapy, CRC Press, Boca Raton

(1994)). Besides high loading capacity, ion-exchange microspheres were also reported to provide sustained drug release profiles so that the exposure of the tumor to the drug can be maximized (Codde et al Anticancer Research. 13(2):539-43 (1993)). In comparison with controls, DOX treatment with free drug, liposomes or microspheres significantly reduced tumor growth by 56% ($P < 0.001$), 51% ($P < 0.01$) and 79% ($P < 0.001$) respectively. Furthermore, the DOX-microsphere treatment was reported to be significantly better than either of the other DOX treatments (53%, $P < 0.05$) or the sham-microsphere treated group (64%, $P < 0.05$).

The effectiveness of chemotherapy has also been limited by the development of a multidrug resistance (MDR) phenotype in cancer patients treated chronically with certain chemotherapeutic agents. One of the major mechanisms of multidrug resistance has been the over-expression of an energy-dependent transport system i.e. P-glycoprotein (P-gp) on tumor cell membranes. Chemosensitizers which reverse MDR usually by interaction with P-gp have been concurrently administered with chemotherapeutic agents to improve chemotherapy. Promising results have been reported in some non-responsive tumors following concurrent administration of a chemotherapeutic agent and a chemosensitizer. However, systemic toxicity is frequently increased with the treatment.

The various drug delivery systems devised for the purpose of chemoembolization have not considered modulation of MDR, nor have they considered reducing the side effects observed with MDR. Therefore, novel formulations are required which combine the advantages of targeted delivery and chemosensitization.

SUMMARY OF THE INVENTION

This invention involves the development of a drug delivery composition containing biodegradable nano- or microspheres which are chemically modified with functional groups to introduce ion-exchange properties to the microspheres, and to modulate hydrophobicity, mechanical strength and drug release profiles. The new system, similar to a polylactic acid/polyglycolic acid system in terms of mechanical strength and drug release profiles, has much higher loading capacity for ionic chemotherapeutic agents like doxorubicin, vinblastine, verapamil, quinidine etc.. Therefore, once used in targeted cancer chemotherapy, it is more efficient as a drug carrier. Additionally, since loading of single or multiple ionic agents can be carried out with ease, for the refractory multi-drug resistant (MDR) cancer phenotypes, the use of this system can achieve simultaneous intratumor delivery of both a chemotherapeutic agent and a chemosensitizer, resulting in improved therapeutic effects and much reduced systemic toxicity. Size control of the new system is easily obtained, facilitating confirmation of clinical utility, and large-scale production.

Broadly stated, the present invention relates to a drug delivery composition comprising microspheres containing at least one chemotherapeutic agent and at least one

chemosensitizer wherein the microspheres have a biodegradable polymer matrix with functional groups which associate with the chemotherapeutic agent and chemosensitizer.

The invention also contemplates a method for preparing microspheres containing at least one chemotherapeutic agent and at least one chemosensitizer comprising:

(a) obtaining microspheres having a biodegradable polymer matrix with functional groups which associates with a chemotherapeutic agent and chemosensitizer; and

(b) mixing the microspheres with at least one chemotherapeutic agent and at least one chemosensitizer.

The invention also provides a method for treating multidrug resistant tumors in a subject comprising administering to the subject an effective amount of a drug delivery composition comprising microspheres containing at least one chemotherapeutic agent and at least one chemosensitizer wherein the microspheres have a biodegradable polymer matrix with functional groups which associate with the chemotherapeutic agent and chemosensitizer.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a is a graph showing the dynamics of verapamil sorption into microspheres through ion exchange with an initial concentration of verapamil at 0.025 mg/ml.

Figure 1b is a graph showing the effect of the microsphere/drug ratio on the equilibrium level of drug loaded and the yield of drug loading with an initial verapamil concentration of 0.05 mg/ml.

Figure 1c is a graph showing competitive loading of vinblastine and verapamil.

Figure 2a is a graph showing fractional release of verapamil and vinblastine from single-agent-loaded microspheres as a function of time.

Figure 2b is a graph showing fractional release of verapamil and vinblastine from dual-agent-loaded microspheres as a function of time.

Figure 3 is a photograph of freeze-dried CMDEX microspheres loaded with doxorubicin. Shown by a confocal fluourescent microscope.

Figure 4 is a graph showing release of doxorubicin from microspheres into a phosphate buffer solution (pH 7.4) at 37 degrees C, as a function of time.

Figure 5 is a graph showing release of verapamil from microspheres in Pluronic F-127 gel into a phosphate buffer solution (pH 7.4) at 37 degrees C, as a function of time.

Figure 6 is a graph showing release of verapamil from hydrophobically modified microspheres in corn oil and from unmodified microspheres in corn oil into a phosphate buffer solution (pH 7.4) at 37 degrees C, as a function of time.

Figure 7 is a graph showing the release of quinidine from CMDEX microspheres and CMDEX microspheres coated with hydrophobic polymers as a function of time.

Figure 8 is a graph showing vinblastine uptake by parent and MDR CHO cells as a function of time.

Figure 9 is a graph which depicts the uptake of doxorubicin by multidrug-resistant murine tumor cells in the presence of chemosensitizing agent, verapamil.

Figure 10 is an IR spectrum of maleic ester of inulin.

Figure 11 is an IR spectrum of inulin.

Figure 12 is an IR spectrum of a copolymer of maleic acid ester of inulin and methacrylic acid.

DETAILED DESCRIPTION OF THE INVENTION

Drug Delivery Composition

As hereinbefore mentioned, the present invention relates to a drug delivery composition comprising microspheres containing at least one chemotherapeutic agent and at least one chemosensitizer wherein the microspheres have a biodegradable polymer matrix with functional groups which associate with the chemotherapeutic agent and chemosensitizer.

The delivery composition of the invention has many other characteristics which make it particularly advantageous. The microspheres used in the delivery system are bio-degradable and are stable in physiological environments. The microspheres using ion-exchange principles exhibit high loading capacity for various chemotherapeutic agents. This allows an effective dose to be used to produce high levels of local drug concentration with consequent greater therapeutic efficacy. Administration of microspheres with high drug loading also results in less of the matrix material being co-administered to the body, and biological reaction to the matrix material is minimized. The microspheres also permit diffusion of the chemotherapeutic agent and chemosensitizer from the core through the matrix at a predetermined release rate. The microspheres can also be sterilized for use before addition of the chemotherapeutic agents and chemosensitizers avoiding degradation of sensitive therapeutics.

Table 1 provides a comparison of the properties of some commercial products and a drug delivery composition of the invention.

The microspheres in the drug delivery composition of the invention comprises a biodegradable polymer matrix. Such biodegradable polymer matrixes may be comprised of polyesters, such as for examples, poly(hydroybutyric acid), poly(hydroxyvalerianic acid-co-hydroxybutyric acid), poly(lactic acid), poly(glycolic acid), poly(lactic acid-co-glycolic acid), poly(ε-caprolactones), poly(ε-caprolactone-co-DL-lactic acid);

polyanhydrides, for example, poly(maleic anhydride); polyamides such as for examples albumin, pol(hydroxyalkyl)-L-glutamines, poly(γ-ehtyl-L-glutaminate-co-glutamic acid), poly(L-leucine-co-L-aspartic acid), poly(proline-co-glutamic acid); poly(orthoesters); poly(alkyl 2-cyanoacrylates); and polysaccharides, such as for examples, starches, glycogen, dextrans, chitin, glucans, fructans like inulin, mannans, xylans, arabinans, galactans, galacturonans, xyloglucans, galatomannans, glucomannans,galactoglucomannans, arabinogalactans, carrageenans, agar, agarose, pectic acids, pectinic acids, alginic acids alginate, gum tragacanth, glycosaminoglycans, hyaluronic acid, chondroitin sulphates, karatan sulphate, dermatan sulphate, and heparin; and including bacterial polysaccharides like lipopolysaccharide, peptidoglycan, teichoic acids, cellulose and xanthan gum. The biodegradable polymer has functional groups which associate with the chemotherapeutic agent and chemosensitizer. The functional groups have the general formula R^1-R^2-polymer where R^1 is an alkyl group, an alkene or alkyne, aryl, alkoxy, or cycloalkyl, preferably a C_1 to C_{10} alkyl, preferably R^1 may be methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, propene, butene, cyclohexene, methylcyclopropyl, methylcyclohexyl, cyclobutyl, or O-methyl; R^2 is a carboxy group (-COOH), a sulfonyl group (-SO3), or -$NR^3R^4R^5$ wherein R^3, R^4, and R^5 are the same or different and represent hydrogen, alkyl, aryl, or cycloalkyl preferably alkyl. R^1 R^3, R^4, and R^5 may contain other chemical functional groups such as halogen, hydroxyl, amine, amide, nitro, and thiol. In preferred embodiments of the invention R^1 is methyl and R^2 is a carboxy group i.e. the functional group is carboxymethyl.

Ionic chemotherapeutic agents are suitable for use in the delivery composition of the invention i.e. either cationic or anionic agents. Examples of ionic agents which may be used in the delivery composition of the invention are alkaloids such as vinblastine and vincristine, antibiotics such as mitomycin C, doxorubicin (adriamycin), daunorubicin, and their derivatives, hormonal agents such as tamoxifen. Other chemosensitzers or G-glycoprotein inhibitors include LY-335979 (Eli Lilly) and GW-918 (GlaxoWellcome).

Ionic chemosensitizers are suitable for use in the delivery composition of the invention. Examples of suitable ionic chemosensitizers which may be used in the delivery system of the invention include calcium channel blockers e.g. verapamil, nifedipine, nicardipine, diltiazem, depridil, felodipine, and their derivatives, calmodulin antagonists e.g. trifluoperazine and chlorpromazine, antibiotics and analogs e.g. cefoperazone and ceftriaxone, indole alkaloids e.g. quinidine, quinine, and quinacrine, and the like.

The biodegradable polymer of the microspheres used in the drug delivery composition of the invention may also have free hydroxyl groups converted to esters. This conversion increases the hydrophobicity of the microspheres. Modification of the free hydroxyl groups results in increased mechanical strength and slows the drug release of the

microspheres in the drug composition. The microspheres can also be coated with hydrophobic polymers to further increase hydrophobicity. Examples of such polymers include poly(methyl methacrylate-co-methacrylic acid), polyurethane, chitin, poly(hydroxyvalerianic acid-co-hydroxybutyric acid), poly(lactic acid), poly(glycolic acid), poly(lactic acid-co-glycolic acid),poly(ε-caprolactones), poly(ε-caprolactone-co-DL-lactic acid); polyanhydrides, e.g., poly(maleic anhydride); polyamides, e.g., albumin, pol(hydroxyalkyl)-L-glutamines,poly(γ-ehtyl-L-glutaminate-co-glutamic acid),poly(L-leucine-co-L-aspartic acid), poly(proline-co-glutamic acid); poly(orthoesters) ; and poly(alkyl 2-cyanoacrylates).

The microspheres in the drug delivery composition of the invention have a diameter of between about 300 microns (μm) and 50 nanometers, and are preferably 5 to 300 μm, most preferably 40 to 200 μm.

The microspheres in the drug delivery composition of the invention may also contain inert excipients commonly used to improve the characteristics of the composition. For example, inert excipients such as vegetable oil, arachis oil, coconut oil, maize oil, almond oil, sesame oil, peanut oil, cottonseed oil, Caster oil, corn oil, olive oil; thermal gels such as Pluoronic series; alcohols like benzyl alcohol, ethanol; syrup; esters such as ethyl oleate, isopropyl myristate, glycerol, propylene glycol, liquid macrogols, esters, may be added to improve viscosity, tonicity, biocompatibility, and release profile and the like. The microspheres may also be dispersed in a physiological medium such as saline.

Process for Preparing Drug Delivery Composition

A method for preparing microspheres containing at least one chemotherapeutic agent and at least one chemosensitizer is provided comprising:

(a) obtaining microspheres having a biodegradable polymer matrix with functional groups which associates with a chemotherapeutic agent and a chemosensitizer; and

(b) mixing the microspheres with at least one chemotherapeutic agent and at least one chemosensitizer.

The microspheres having a biodegradable polymer e.g. albumin or dextran, may be prepared using conventional methods or they may be obtained from commercial sources. For example, commercial cross-linked dextran microspheres (Sephadex G-10, G-25, G-50, G-100 and G-200) may be obtained from Pharmacia. Preferably the microspheres have a particle size of about 40-200 μm and a selected pore size range for molecules with molecular weight of 100-600,000 Da. The microspheres are chemically modified to introduce the functional groups. By way of example, carboxymethylation or sulphonation may be carried out to introduce a carboxymethyl group or a sulfonyl group, respectively. In an embodiment, carboxymethylated dextran ion exchange microspheres are prepared by suspending dextran gel (about 2 to 5 grams, preferably 3 grams) in about 40 wt% sodium

hydroxide and adding chloroacetic acid (about 10 to 20 grams, preferably 16 grams). Anionic carboxylic groups (1.95 meq/g) may also be introduced by a base-catalyzed reaction with succinic anhydride.

The present inventors have found that drug loading of the chemotherapeutic agent and chemosensitizer is proportional to the extent of functional modification of the polymer matrix. Generally, drug loading increases with increased modification of the microspheres. In an embodiment of the invention, at least 10% of the hydroxyl groups on the polymer matrix are modified to provide for at least 50% loading of the two drugs.

Free hydroxyl groups on the prepared microspheres may be converted to esters, or ethers or otherwise blocked, by, for example, introducing fatty groups to OH groups via an ester bond, ether bond,amide bond, imide bond. It is also possible to increase hydrophobicity by introducing hydrophobic polymers by copolymerization or grafting/block copolymerization or introducing cross-linking agents. Such further modification of the free hydroxyl groups results in increased mechanical strength and slows the drug release of the microspheres in the drug composition.

It will be appreciated that the polymer matrix of the microspheres will be modified to a degree to provide an appropriate loading, and release for the particular combination of chemotherapeutic agent and chemosensitizer and to suit a particular therapeutic application.

Free flowing drug loaded microspheres may be obtained after incubation of the prepared microspheres in distilled water or a polar organic solvent, such as ethanol or methanol (for hydrophobic drugs such as cyclosporin DMSO may be used), containing the chemotherapeutic agent and chemosensitizer overnight, followed by filtration, washing with distilled water, and freeze-drying. Chromatographic methods may also be used to enhance loading of the chemotherapeutic agent and chemosensitizer.

As discussed above, inert excipients commonly used to improve the characteristics of the composition such as thickeners, surfactants etc. may be added to the microspheres to improve viscosity, tonicity, biocompatibility, stability and the like. In addition, lubricants, dyestuffs, sweetners, flavouring agents, inert excipients, preservatives etc. may be added to the microspheres to improve and permeability properties. The microspheres may also be dispersed in a physiological medium such as saline.

The microspheres may be sterilized e.g. by heat or UV light before addition of the chemotherapeutic agents and chemosensitizers. The agents and chemosensitizers may be sterilized separately avoiding possible degradation of sensitive therapeutics.

A time release profile for the drugs can be optimized taking into consideration the amount of drug loaded into the drug delivery composition, the solubility of the drug in the surrounding physiological milieu the affinity of the drug to the microspheres, the

hydrophobicity of the microspheres and the coating, and the particle size and size distribution of the microspheres in the composition.

Applications

The drug delivery composition of the invention exhibits a sustained drug release profile, and it provides a chemosensitization effect in drug resistant cells. Accordingly, the drug delivery system may be used in the treatment of multidrug drug resistant tumor cells. It is expected that the drug delivery compositions will be particularly useful in the treatment of malignancies including multiple myeloma, breast cancers, ovarian cancers, childhook neuroblastoma, leukemia, pancreas carcinomas, liver carcinomas, cervical carcinoma, endometrial carcinomas, and adenocarcinomas of the kidney and colon. The drug delivery composition may also be used to deliver other combination drug therapies, for examples, AZT and 3TC (anti-AIDS); angiostatin and endostatin (anticancer); Intro-A (Schering-Plough) and Ribavirin (ICN) (Hepatitis C treatment); anticancer drugs (e.g. 5-FU) + vasoconstrictors (e.g.epinephrine); anticancer drugs (e.g. taxol)+ chemosensitizers (e.g. cyclosporins). *In vitro* and *in vivo* model systems may be used to assess the therapeutic efficacy and system. For example, an in vitro system using multidrug resistant CH^RC5 CHO cells may be used to test therapeutic efficacy in multidrug resistant tumors.

Therefore, the present invention contemplates a method for treating multidrug resistant tumors in a subject comprising administering to the subject an effective amount of a drug delivery composition comprising microspheres containing at least one chemotherapeutic agent and at least one chemosensitizer wherein the microspheres have a biodegradable polymer matrix with functional groups which associate with the chemotherapeutic agent and chemosensitizer.

The drug delivery compositions may be delivered to a target site through a variety of known routes of administration. For example, a drug delivery composition comprising cross-linked dextran microspheres for use in treating a multidrug resistant tumor may be administered by intratumor injection. Other administration routes include oral deliver for verapamil and other weak acidic or basic drugs; rectal delivery and topical adminstration such as creams for dermal or transdermal application, ophthalmic application, containing drugs such as actibiotics, antifungal agents, anticancer drugs, antiglucoma agents, localanesthetic agents, anti-inflammatory, analgesic agents.

The drug delivery compositions of the invention can be intended for administration to humans or animals. Dosages of the chemotherapeutic agent and chemosensitizer incorporated in the drug delivery composition will depend on individual needs, on the desired effect and on the chosen route of administration.

In yet another application, the microspheres loaded with chemosensitizers and radiolabeled P-glycoprotein substrates (e.g. vinblastine, vincrestine, taxol and sestamibi

are injected intratumorally, among which sestamibi is preferable because it is not a cytotoxic agent. Radio image of the tumor gives the concentration of the radiolabeled chemicals in the tumor. The MDR tumors will show low concentration of the chemicals in the absence of chemosensitizers, whereas the non-resistant tumors higher concentration. More importantly, in the presence of microsphere-delivered chemosensitizers, the increment of concentration of the radiochemicals will be much higher in the MDR tumors than that in the non-resistant tumors.

The following non-limiting examples are illustrative of the present invention:

Examples

Example 1

Synthesis and Characterization of Dextran Ion Exchange Microspheres

a) Carboxymethylation of Dextran Microspheres (DMDEX/MS)

Cross-linked dextran gels (Sephadex G-200) are used to prepare carboxymethylated dextran ion exchange MS drug carriers. In a typical preparation, 3 g of dextran gel were suspended in 50 ml of 40 wt.% sodium hydroxide solution and 16 g of chloracetic acid were then added to this suspension. The reaction mixture was gently agitated for 12 hours at room temperature. The reaction mixture was washed extensively with distilled water and then freeze dried. The content of ion exchange carboxymethyl groups was assayed by acid-base titration method.

b) Drug Loading and Release Studies

Drug loading is carried out by mixing drug solutions in distilled water with freeze-dried resins. In a typical preparation, 0.1 g of ion exchange resin was added to 10 ml of 1% verapamil or doxorubicin aqueous solution. After overnight incubation, the resin was isolated by either centrifugation or filtration followed by extensive washing with distilled water and then lyophilization. Unbound drug in the wash was determined by UV/VIS spectroscopy (HP8452A). For drug release experiments, the drug-loaded resins were added to buffer solution directly, or incorporated into other pharmaceutical vehicles first, and then added to the buffer solution. The pharmaceutical vehicles include aqueous system such as thermal gels and hydrocarbon system such as sesame oil, vegetable oil, and corn oil. UV/VIS spectroscopy was applied to assay the drug released from the delivery system into the buffer solution. When more than one compound (i.e., doxorubicin or vinblastine and verapamil) is used for simultaneous loading and release, the same loading procedure is followed but the different compounds are analyzed by high performance liquid chromatography (HPLC).

In a typical loading process, 0.05g of the dry, ionic microspheres (e.g. MG-50) were added to 10 ml of 0.5% verapamil aqueous solution. After incubation at room temperature for predetermined time intervals,

the microspheres were separated by centrifugation, and the drug concentration in the supernatant was analyzed by UV/VIS spectrophotometer (HP 8452A) at a wavelength of

270 nm for vinblastine and 278 nm for verapamil. Drug loading was calculated from the difference between the amount of drug originally used and that after incubation with the ion exchange microspheres. In the case of loading of dual agents, e.g., vinblastine and verapamil, the same procedures were applied except for that the drug assay was carried out by HPLC (Waters). The mobile phase consisted of phosphate buffer (ionic strength 0.1 M, pH7.0), tetrahydrofuran, and methanol with a volume ratio of 0.43:0.41:0.16. Vinblastine and verapamil were separated in a reverse-phase column (Norva-pak C-18, Waters) by the mobile phase at a flow rate of 0.6 ml/min. Their retention time was 5.94 and 6.82 min., respectively, as measured by a UV detector at a wavelength of 270 nm.

Curve A in Figure 1a depicts the fraction of remaining verapamil in the solution as a function of time for the microspheres incubated in 0.025 mg/ml verapamil solution. A rapid decrease in the remaining drug is observed in the initial 10 hours followed by a slower change in the subsequent 10 hours. A plateau is reached after 20 hours indicating an equilibrium state. Similar trend is also observed with vinblastine. Therefore, 30 hours of incubation was carried out for all drug loading to ensure completion of the process. Curve B in Figure 1a shows that the fraction of drug loaded into the microspheres follows a typical first-order sorption kinetics, suggesting that the drug loading is essentially a diffusion-controlled process like drug release.

The amount of microspheres relative to drug is an important factor influencing the equilibrium drug content and the yield of drug loading. However, there has been little systematic work in this area. The yield of drug loading and the equilibrium level of verapamil loaded are plotted in Figure 1b against the ratio of the microspheres to the drug (M/D ratio). As the M/D ratio increases, the yield of drug loading increases while the equilibrium level of drug loaded decreases. This indicates that, in order to raise the equilibrium drug content, one has to sacrifice the loading efficiency. Therefore, the compromise approach is to control the conditions in the left region, e.g., M/D ratio between 1 and 3. In this case, the drug content reaches ~30% with the yield of drug loading 40-60%.

Competitive loading of dual agents is an indication of relative affinity of the drugs to the microspheres which is related to the relative release rate. Figure 1c shows that the relative amount of vinblastine in the microspheres is higher than verapamil. This explains partly why vinlastine is released more slowly than verapamil.

c) Drug release from microspheres in a buffer solution

At predetermined time intervals, the suspension was centrifuged and the supernatant was analyzed spectroscopically or chromatographically. Figure 2a shows the fractional release of verapamil and vinblastine from individually-loaded microspheres. Figure 2b depicts the release profiles of verapamil and vinblastine from dual-agent-loaded microspheres. Both graphs indicate that the drugs are released for a prolonged period of time and the release rate of verapamil is higher than that of

vinblastine. Figure 3 is a confocal fluorescent photograph of doxorubicin-loaded microspheres. Figure 4 illustrates the sustained release of doxorubicin from the microspheres into pH 7.4 buffer solution at 37 °C.

d) Drug release from microspheres in a synthetic thermal gel (Pluronic F-127)

An aqueous solution (20-30%) of Pluronic F-127 was prepared at 4 °C. This solution experiences gelation at temperatures higher than 10 °C. Microspheres (Sephadex SP C-25) loaded with ionic drug(s) (e.g., verapamil or doxorubicin) were added to the cold solution under stirring. The suspension showed the same reversible thermal gelation as the parent Pluronic F-127 solution, as demonstrated in heating and cooling cycles. It was syringe-injectable at lower temperature. Therefore, once localized at sites of physiological conditions, it became semi-solid gel. The gel, well tolerated by tissue itself, could prevent the microspheres from coagulation and slow down the drug release rate. In vitro release from the above mixture was demonstrated by transferring 1 g of the gel with 5 wt.% of microspheres loaded with 29 wt.% verapamil into a dialysis tubing (molecular weight cut-off: 6000). Drug release from the clamped tubing in 200 ml phosphate buffer (0.05 M, pH 7.4) was monitored with UV absorbance at 278 nm. Figure 5 displays the fractional release of verapamil from the ion exchange resin in Pluoronic F-127 into pH 7.4 buffer at 37 °C which is significantly slower than that directly contact with the buffer solution (see Figure 2a).

e) Drug release from microspheres in vegetable oil

Microspheres loaded with ionic drug (i.e., verapamil or doxorubicin) were added to corn oil to provide a syringe-injectable oil formulation. The oil phase behaved as a barrier for the ion exchange process required for drug release. Therefore, slower release was obtained. Hydrophilic polysaccharide microspheres (e.g., SP C-25) and their corresponding hydrophobically-modified product (SP C-25/palmitoyl cholride) were tested for their release profiles. The hydrophobically-modified product mixed well in the oil while the unmodified one coagulated. Release experiments were undertaken using the same method described above. The release profiles are presented in Figure 6.

f) Modification of the Resin Hydrophobicity for Better Control in Drug Release Kinetics

Hydrophobic resins, therefore less swellable in water and probably with higher affinity for hydrophobic drugs (such as digoxin, taxol, cyclosporins, nifedipine, cisplatin, pentaerythritoltetranitrate, indomethacin, theophylline, AZT, and cipro) by esterification of the ion exchange resins and/or by coating with hydrophobic polymers without jeopardizing the ion exchange capacity. Typically, 3 g of CMDEX/MS was added to 100 ml of DMSO containing 15 g of acetyl chloride as esterification agent and pyridine as catalyst. After overnight reaction, the mixture was filtered and the resin was washed first with organic solvent and then with distilled water. In another example, 15 g of palmitoyl chloride in the place of acetyl chloride was used to react with 3 g of ion

exchange resin (SP-C25) in 100 ml of DMSO with triethylamine as the catalyst. The same reaction condition and purification procedures were employed. More hydrophobic resins were prepared by coating the esterified microspheres with hydrophobic polymers such as poly(lactic-co-glycolic acid). Free-flowing microspheres of lower degree of swelling in water were finally obtained by freeze-drying.

Figure 6 illustrates the results of a comparison of the release of verapamil from hydrophobically-modified resin in oil vehicle with that without modification. Much slower release of the drug was realized from the hydrophobically-modified resin. Figure 7 shows the release of quinidine from CMDEX/MS alone (without coating) and CMDEX/MS coated with hydrophobic polymers (with coating). Again, the results demonstrate that surface coating of the microspheres with hydrophobic polymers significantly reduced the drug release rate.

Example 2

In vitro studies of drug accumulation of vinblastine by Chinese hamster ovary (CHO) cells

a) Tissue cell culture and accumulation of 3H-vinblastine by parent and multidrug resistant cells in presence of chemosensitizer

Parent (AuxB1) and multidrug resistant (CHRC5) Chinese hamster ovary (CHO) cells, originally selected from the parent line for resistance to colchicine (180 times relatively resistant to colchicine and 30 times to vinblastine) are grown in 25 cm2 and 75 cm2 plastic tissue culture flasks in alpha minimal essential medium (a-MEM), containing 10% fetal bovine serum and 0.5% penicillin-streptomycin at 37 °C in an atmosphere of air and 5% CO2.

For the accumulation studies, a similar methodology as described before (Bondanan, J. et al. *Am. Soc. Nephrol.* 5:75-84 (1994)) was used. Cells were grown on 24 well plates for 3-4 days until they reached confluence. Drug accumulation was initiated by the addition of 0.5 ml of Earle Balanced Salt Solution (EBSS) containing 21 nM (1/3 3H-labelled and 2/3 cold) vinblastine sulfate and ^{14}C-mannitol (an extracellular marker). Cells were then incubated for 0 to 2 h at 37 °C in an atmosphere of air + 5% CO2. Drug accumulation by the cells was rapidly stopped by aspirating the media and by washing the cells twice with 2 ml of ice-cold 0.16N NaCl. The cells were then lysed with 1 N NaOH, followed by neutralisation with 2 N HCl, and then counted in a liquid scintillation counter. Protein determination was performed by a standard colorimetric method well know to those skilled in the art. The accumulation of vinblastine by the cells was corrected for the extracellular binding as determined by the accumulation of ^{14}C-mannitol.

Figure 8 shows the uptake of vinblastine over time for both parent AuxB1 (series 2) and multi-drug resistant CHRC5 CHO cells (series 1). As can be seen from Figure 8 the uptake of vinblastine is greater in the parent cells as compared to the MDR cells. This

demonstrates that the CHRC5 CHO cells used in this experiment are in fact multi-drug resistant cells.

Initially the accumulation of vinblastine over time by the parent and resistant CHO cells is determined in the presence and absence of chemosensitizers (i.e., 20mM and 50mM cyclosporin A, 50mM quinidine, 50mM verapamil) to verify that the cells are retaining MDR properties. Then CHRC5 cells are used to evaluate the effectiveness of vinblastine and/or verapamil loaded into the microspheres. The amounts of loading are controlled to make sure that final concentrations of vinblastine and/or verapamil are similar to conditions using free agents.

Table 2 shows the uptake of vinblastine by the multi drug resistant CHO cells in the presence of immobilized verapamil as compared with free agents and placebo microspheres. At one hour the uptake of vinblastine is 0.69±0.07 for CMDEX/MS beads loaded with vinblastine as compared to 6.81±0.43 for CMDEX/MS beads loaded with vinblastine and verapamil. At two hours the uptake of vinblastine is 1.05±0.09 for CMDEX/MS beads loaded with vinblastine as compared to 8.05±0.64 for CMDEX/MS beads loaded with vinblastine and verapamil. These results demonstrate that the loading of vinblastine is enhanced approximately 9.9 times at one hour and 7.7 times at 2 hours when the microspheres have been loaded with verapamil.

b) Cell viability-Trypan blue test

Viability of multidrug resistant (CHRC5) Chinese hamster ovary cells in the presence chemosensitizers or ion exchange resins is determined according to the standard Trypan blue test.

The cytotoxicity of CMDEX/MS or its acetylated derivative AC-CMDEX/MS was investigated. The results shown in Table 3 demonstrate that CMDEX/MS and AC-CMDEX/MS were not noticeably toxic as compared to a control.

Example 3

In vitro studies of drug accumulation of doxorubicin and vinblastine by murine tumor cells

Murine breast sarcoma cell line EMT6/P (parent) and the resistant variant EMT6/AR1.0 are used as the model system. The latter was selected by exposure to doxarubicin and overexpression of P-gp. Cells are grown in alpha minimum-essential medium (a-MEM) with 10% fetal bovine serum and 0.1 mg/mL kanamycin.

When EMT6/P and EMT6/AR1.0 cells are grown in multiwell plates for 3-4 days, reaching sub-confluence, drug accumulation is initiated by the addition of 0.5 mL a-MEM/30 mM HEPES containing 14C-doxorubicin and ^{3}H-mannitol (an extracellular marker). After incubation for 0-120 minutes, cellular accumulation is rapidly stopped by aspirating the medium and by washing the cells twice with 1 mL ice-cold 0.9% NaCl. The cells are lysed with 0.5 N NaOH followed by neutralization and then counted in a liquid scintillation counter. The accumulation of ^{14}C-doxorubicin by the cells is corrected

for extracellular binding as determined by the accumulation. The effect of immobilized chemosensitizer and/or cytotoxic agent on drug accumulation is investigated and compared with placebo and free chemosensitizers and free cytotoxic agent.

In the studies of vinblastine accumulation by the murine cells, the same procedures are employed as in the studies of doxorubicin except that ^{3}H-vinblastine and ^{14}C-mannitol are used.

Figure 9 depicts the uptake of doxorubicin by the multidrug-resistant murine tumor cells in the presence of chemosensitizing agent, verapamil, is increased more than 2.6 fold. Based on these results and the in vitro release rate of doxorubicin (Figure 4) and verapamil (Figure 2a), the cellular uptake of doxorubicin loaded in the microspheres is expected to increase to the same extent in the presence of verapamil. A slower uptake kinetics may be anticipated because of slow release of doxorubicin from the microspheres.

Example 4

In vivo therapeutic trials using mice and murine tumor cells

Parent and multidrug resistant murine tumor cells, EMT6/P and EMT6/AR1.0, are grown in monolayer using the same method described in Example 3. The cells are released from the plates with trypsin and resuspended for injection into mice. In vivo growth is initiated by intramuscular injection of 1x106 cells into the left hind leg of 8-12 week old syngeic Balb/c mice weighing 25-30 g. Growth of tumors is monitored daily by passing the tumor-bearing leg through graded holes in a strip of Lucite and the leg diameter is converted to an estimate of tumor weight using a previously defined calibration curve. When mouse tumors have reached a size of 0.6-0.9 g, the therapy is initiated. Four groups of 6 mice for each of the two tumor types (EMT6/P and EMT6/AR1.0) are used for the therapeutic trials. Each treatment involves intratumoral injection in a volume of 50-100 mL containing 10-25 mg chemotherapeutic agent and chemosensitizer. Group C serves as control and receives placebo microspheres. Group D receives doxorubicin-loaded microsphere, Group V receives verapamil-loaded microsphere, and Group DV receives both doxorubicin- and verapamil-loaded microspheres. Tumor growth is monitored daily and the mice are sacrificed when tumors reach an estimated size of 1.5 g (about 6% of body weight). The dosages of doxorubicin and verapamil is determined from in vitro data and preliminary in vivo experiments.

Tumor response to therapy is assessed using the growth curves and the delay in growth of tumors indicates therapeutic response.

Example 5

Synthesis and characterization of microspheres of maleic ester of inulin and methacrylic acid

Preparation of maleic ester of Inulin

5 g of inulin was dissolved in 200 ml of warm water (~40 °C). 12 g of maleic anhydride and 3 g of sodium acetate were added. The reaction mixture was stirred for 24 hours at 40 °C. The reaction product was isolated by precipitation in a 4-fold volume of anhydrous ethyl ether. The precipitates were washed with ice cold water, dissolved in an aqueous solution of sodium bicarbonate. The sodium salt of the product was precipitated by addition of acetone, filtered, and then dissolved in water. Further purification was undertaken by precipitating the free ester with hydrochloric acid, washed with ice cold water (0-5 °C), and then dissolved in 4:1 (V/V) mixture of acetone and isopropyl alcohol. The product was finally separated by addition of ether. The resultant maleic half ester of inulin was a white powder, soluble in methanol, ethanol, DMF, swollen by cold water, soluble in warm water, and insoluble in ether.

Characterization of maleic ester of Inulin

Infra red spectra

Figure 9 shows the FTIR spectrum of maleic half ester of inulin in comparison with that of inulin (Figure 10). C-O stretching band, i.e., 1100-1300 cm^{-1} in the ester bond is clearly seen in the figure, which is absent in Figure 10. The presence of maleic ester is indicated by strong C=O stretching absorption band in the region of 1730 cm^{-1} and that of olefinic double bond in conjugation with carbonyl group at 1640 cm^{-1} indicate. The broad and intense O-H stretching absorption at ~3350 cm^{-1} in inulin is significantly reduced after esterification.

Copolymerization of inulin half ester of maleic acid with methacrylic acid

Copolymerization was carried out in distilled water at 70 °C under nitrogen atmosphere using N,N-methylene bisacrylamide (BIS) as the cross linking agent and potassium persulfate (KPS) as the initiator. To monomer mixture containing maleic half ester of inulin and inhibitor-free methacrylic acid to a total concentration of 1.82 mole, 10% BIS was added and then polymerization was initiated by the addition of small amount of concentrated KPS 0.05 mole solution. After polymerization for 4 hours, the copolymer was separated and washed several times with distilled water to remove the impurities.

Infra red spectra

Figure 11 is the IR spectrum of the copolymer of maleic ester of inulin and methacrylic acid which was synthesized without using cross-linking agent. It shows absence of olefinic double bond at ~1620 cm^{-1} (refer to Figure 9) indicating that the double bonds of maleic half ester of inulin are involved in copolymerization.

Solubility of the copolymer

The solubility of the copolymer and inulin ester was tested in order to verify the incorporation of the monomer into the copolymer. The results are shown in Table 4 together with the solubility of the monomers. Taking into account that monomers are

soluble in water, DMF, and methanol, the solubility change indicates that most of monomers have been incorporated into the copolymer.

Degree of swelling

The degree of swelling of the cross-linked copolymer of maleic half ester of inulin and methacrylic acid was determined by allowing the copolymer sample to equilibrate in distilled, deionized water at room temperature. The mass of the swollen sample was determined by blot-and-weigh technique. Dry copolymer mass was determined by drying the sample at room temperature and then in vacuum at room temperature. The degree of swelling was then calculated by the ratio of the weight of swollen copolymer to that of dry copolymer.

Preparation of microspheres of maleic ester of inulin and methacrylic acid

The microspheres of maleic ester of inulin and methacrylic acid were prepared by suspension polymerization. Aqueous solution of inulin ester, methacrylic acid, and N,N-methylene bisacrylamide was dispersed in oil phase by vigorous stirring. In a typical polymerization, 10 ml of the aqueous solution was added to 100 ml of paraffin oil containing 10% (v/v) nonionic surfactants, e.g., Pluoronic L-62 and Span 80 with a volume ratio of 3:1. The polymerization was initiated at 70 °C under nitrogen atmosphere by addition of potassium persulfate as the initiator. After polymerization for 6 hours, the microspheres were filtered and washed with distilled water to remove the impurities. The purified microspheres containing ion exchange groups, $-COO^-$, were subject to the tests of drug loading and release, in vitro evaluation of drug uptake, and in vivo therapeutic trials using the same methods as for dextran-based microspheres.

Example 6

Sustained retention of doxorubicin in murine solid tumors

The mouse tumors were grown using the method described in Example 4. When the tumors reached a size of 0.6-0.9 g, 50-100 mL of microsphere suspension was injected into the tumor. Typically, 50 mL of the suspension containing 10 mg/μL microspheres loaded with 60% doxorubicin was administered. The mice were sacrificed at various time intervals (e.g., 3 hours, 1 day, and 3 days) and the tumors were taken and immersed in liquid nitrogen and then in formaline to fix the texture of the tissue. The tumors were cut into thin slices which were then subject to confocal fluorescence imaging. The fluorescent image of doxorubicin was obtained using confocal fluorescent microscopy (MRC 600) with an excitation wavelength of 488 nm and an emission wavelength longer than 515 nm. Based on personal observation, the tumors containg the microspheres were still releasing doxorubicin.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to

cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Table 1. Comparison of Commercial Products and Drug Delivery Composition of the Invention

Ion-exchange Microspheres	High capacity	Drug Release Time	Mechanical Strength	Biodegrad-ability	Size Control	Hydrophobicity
Poly-styrene	Y	Y	Y	N	Y	Y
Cross-linked Dextran	Y	N	N	Y	Y	N
PGA/PLA*	N	Y	Y	Y	N	Y
Our System	Y	Y	Y	Y	Y	VARIES

*Represents microspheres made in situ for controlled release formulation.

Table 2. Vinblastine uptake by CHRC5 cells in the presence of chemosensitizers

Time	Sample	Vinblastine Uptake (pmole/mg protein/ml)	Ratio of Enhancement
1 h	Control	1.56±0.11	
	Blank Beads[1]	1.02±0.05	0.65
	Verapamil(50uM)	5.81±0.52	3.7
	Ver(50uM)+BB1	5.78±0.69	3.7
	VEbead[2]	5.09±0.61	3.3
	Cys A(20uM)	12.77±1.36	8.2
	Cys A(20uM)+BB[1]	12.40±1.32	7.9
	MS1[3]	0.69±0.07	0.44
	MS2[4]	6.81±0.43	4.4
	MS2/MS1		9.9
2 h	Control	2.08±0.09	
	Blank Beads[1]	1.16±0.19	0.56
	Verapamil(50uM)	6.12±0.55	2.9
	Ver(50mM)+BB[1]	6.54±0.24	3.1
	VEbead[2]	5.56±0.40	2.7
	Cys A(20uM)	13.5±1.62	6.5
	Cys A(20uM)+BB[1]	14.2±1.98	6.8
	MS1[3]	1.05±0.09	0.51
	MS2[4]	8.05±0.64	3.9
	MS2/MS1		7.7

1. Placebo - blank CMDEX/MS beads (1% wt./v) were added into transport medium, 50mM verapamil or 20mM Cys A.
2. CMDEX/MS beads (1% wt./v) loaded with verapamil.
3. CMDEX/MS beads (1% wt./v) loaded with vinblastine.
4. CMDEX/MS beads (1% wt./v) loaded with vinblastine and verapamil.

Table 3. Cytotoxicity of CMDEX/MS and its acetylated derivative (AC-CMDEX/MS)

Sample	Cell Viability (Nonviable/Viable, %)	
	1 Hour Incubation	3 Hour Incubation
Control	8±2	19±4
CMDEX/MS	7±1	21±6
AC-CMDEX/MS	8±2	20±4

Table 4. Solubility behavior of monomers and copolymer

	Methanol	DMF	Water
MAA	+	+	+
Maleic half ester of inulin	+	+	+
Copolymer	-	-	-*

+ soluble; - insoluble, * swelling.

We Claim:

1. A drug delivery composition comprising microspheres containing at least one chemotherapeutic agent and at least one chemosensitizer wherein the microspheres have a biodegradable polymer matrix with functional groups which associate with the chemotherapeutic agent and chemosensitizer.

2. The drug delivery composition of claim 1 where the biodegradable polymer is a polysaccharide selected from the group consisting of dextran, inulin, starch, chitin, glycogen, glucans, fructans, mannans, xylans, arabinans, galactans, galacturonans, xyloglucans, galatomannans, glucomannans, galactoglucomannans, arabinogalactans, carrageenans, agar, agarose, pectic acids, pectinic acids, alginic acids alginate, gum tragacanth, glycosaminoglycans, hyaluronic acid, chondroitin sulphates, karatan sulphate, dermatan sulphate, heparin, bacterial polysaccharides, peptidoglycan, teichoic acids, cellulose and xanthan gum.

3. The drug delivery composition of claim 1 where the biodegradable polymer is a polyester selected from the group consisting of, poly(hydroybutyric acid), poly(hydroxyvalerianic acid-co-hydroxybutyric acid), poly(lactic acid), poly(glycolic acid), poly(lactic acid-co-glycolic acid), poly(e-caprolactones), and poly(e-caprolactone-co-DL-lactic acid).

4. The drug delivery composition of claim 1 where the biodegradable polymer is a polyanhydride.

5. The drug delivery composition of claim 4 where the polyanhydride is poly(maleic anhydride).

6. The drug delivery composition of claim 1 where the biodegradable polymer is a polyamide selected from the group consisting of, albumin, pol(hydroxyalkyl)-L-glutamines poly(γ-ehtyl-L-glutaminate-co-glutamic acid), poly(L-leucine-co-L-aspartic acid), and poly(proline-co-glutamic acid).

7. The drug delivery composition of claim 1 where the biodegradable polymer is a poly(orthoester) or poly(alkyl 2-cyanoacrylate).

8. The drug delivery composition of claim 1 where the functional groups have the general formula R^1-R^2-polymer, wherein R^1 is an alkyl, alkene, alkyne, aryl, alkoxy, cycloalkyl, halogen, hydroxyl, amine, amide, nitro, or thiol and R^2 is a carboxy

group (-COOH), a sulfonyl group ($-SO_3$), or $-NR^3R^4R^5$ wherein R^3, R^4, and R^5 are the same or different and represent hydrogen, alkyl, aryl, cycloalkyl, halogen, hydroxyl, amine, amide, nitro, or thiol.

9. The drug delivery composition of claim 8 where R^1 is selected from the group consisting of methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, propene, butene, cyclohexene, methylcyclopropyl, methylcyclohexyl, cyclobutyl, and O-methyl.

10. The drug delivery composition of claim 8 where R^2 is $-NR^3R^4R^5$ and R^1, R^3, R^4, and R^5 are selected from the groups consisting of halogen, hydroxyl, amine, amide, nitro, and thiol.

11. The drug delivery composition of claim 8 where R^1 is methyl and R^2 is a carboxy group.

12. The drug delivery composition of claim 11 where R^2 is carboxymethyl.

13. The drug delivery composition of claim 1 where the polymer is dextran.

14. The drug delivery composition of claim 13 where the dextran is carboxymethylated.

15. The drug delivery composition of claim 14 where the chemotherapeutic agent is vinblastine and the chemosensitizer is verapamil.

16. A drug delivery composition comprising microspheres containing vinblastine and verapamil, wherein the microspheres are a dextran polymer matrix with functional groups of the general formula R^1-R^2-polymer, wherein R^1 is methyl and R^2 is carboxymethyl, which functional groups associate with the vinblastine and verapamil.

17. The drug delivery composition of any one of claims 1 to 16 wherein the microspheres have a diameter of about 300 microns (μm) to about 50 nanometers.

18. The drug delivery composition of claim 17 wherein the microspheres have a diameter of about 5 microns to about 300 microns

19. The drug delivery composition of claim 18 wherein the microspheres have a diameter of about 40 microns to about 200 microns.

20. The drug delivery composition of claim 1 wherein the chemotherapeutic agents are cationic or anionic agents.

21. The drug delivery composition of claim 20 wherein the chemotherapeutic agent(s) is/are selected from the group consisting of alkaloids, antibiotics and hormonal agents.

22. The drug delivery composition of claim 21 wherein the alkaloid is vinblastine or vincristine, the antibiotic is mitomycin C, doxorubicin (adriamycin), or daunorubicin, and the hormonal agent is tamoxifen.

23. The drug delivery composition of claim 1 wherein the ionic chemosensitizers are calcium channel blockers, calmodulin antagonists and analogues thereof, antibiotics and analogs thereof, or indole alkaloids.

24. The drug delivery composition of claim 23 wherein the calcium channel blocker is one of verapamil, nifedipine, nicardipine, diltiazem, depridil, felodipine, the calmodulin antagonist is either trifluoperazine or chlorpromazine, the antibiotic is cefoperazone or ceftriaxone, and the indole alkaloid is one of quinidine, quinine, or quinacrine.

25. The drug delivery composition of claim 1 wherein the biodegradable polymer has free hydroxyl groups converted to esters.

26. The drug delivery composition of claim 25 wherein the microspheres are coated with at least one hydrophobic polymer.

27. The drug delivery composition of claim 26 wherein the at least one hydrophobic polymer is selected from the group consisting of poly(methyl methacrylate-co-methacrylic acid), polyurethane, chitin, poly(hydroxyvalerianic acid-co-hydroxybutyric acid), poly(lactic acid), poly(glycolic acid), poly(lactic acid-co-glycolic acid),poly(ε-caprolactones), poly(ε-caprolactone-co-DL-lactic acid); polyanhydrides; polyamides; poly(orthoesters) ; and poly(alkyl 2-cyanoacrylates).

28. The drug delivery composition of any one of claims 1 to 27 wherein the microspheres contain at least one inert excipient.

29. The drug delivery composition of claim 28 wherein the at least one inert excipient is selected from the group consisting of glycerol, propylene glycol, liquid macrogols, sesame oil, peanut oil, cottonseed oil, Caster oil, corn oil, olive oil, and esters.

30. A method for preparing microspheres containing at least one chemotherapeutic agent and at least one chemosensitizer the method comprising:

(a) obtaining microspheres having a biodegradable polymer matrix with functional groups capable of associating with the chemotherapeutic agent and chemosensitizer; and

(b) contacting the microspheres with at least one chemotherapeutic agent and at least one chemosensitizer.

31. The method of claim 30 wherein the contacting is achieved by mixing.

32. The method of either claim 30 or 31 wherein the step of contacting the microspheres with at least one chemotherapeutic agent and at least one chemosensitizer is preceded by sterilization of the microspheres and the chemotherapeutic agent and chemosensitizer.

33. The method of claim 30 wherein the microspheres are dextran and the functional groups have been introduced by carboxymethylation or sulphonation.

34. The method of claim 33 with the further step of converting free hydroxyl groups to esters being carried out before the step of contacting the microspheres with at least one chemotherapeutic agent and at least one chemosensitizer.

35. A method for treating multidrug resistant tumors in a subject comprising administering to the subject an effective amount of a drug delivery composition according to any one of claims 1 to 29.

36. A use of a drug delivery composition according to any one of claims 1 to 29 to treat a multidrug resistant tumour.

FIGURE 1A

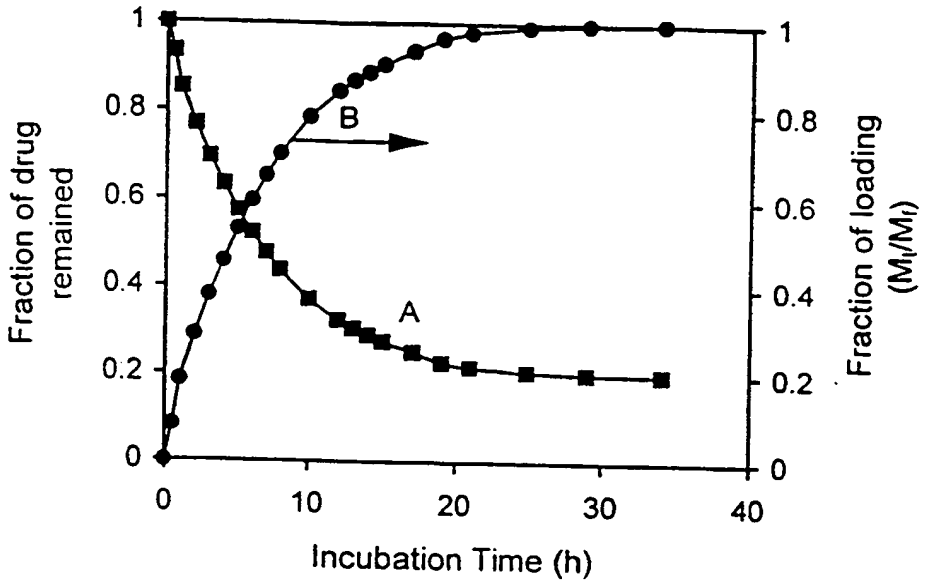

Fraction of drug remained
Fraction of loading (M_t/M_f)
Incubation Time (h)
A
B
0
0.2
0.4
0.6
0.8
1
0
10
20
30
40

FIGURE 1B

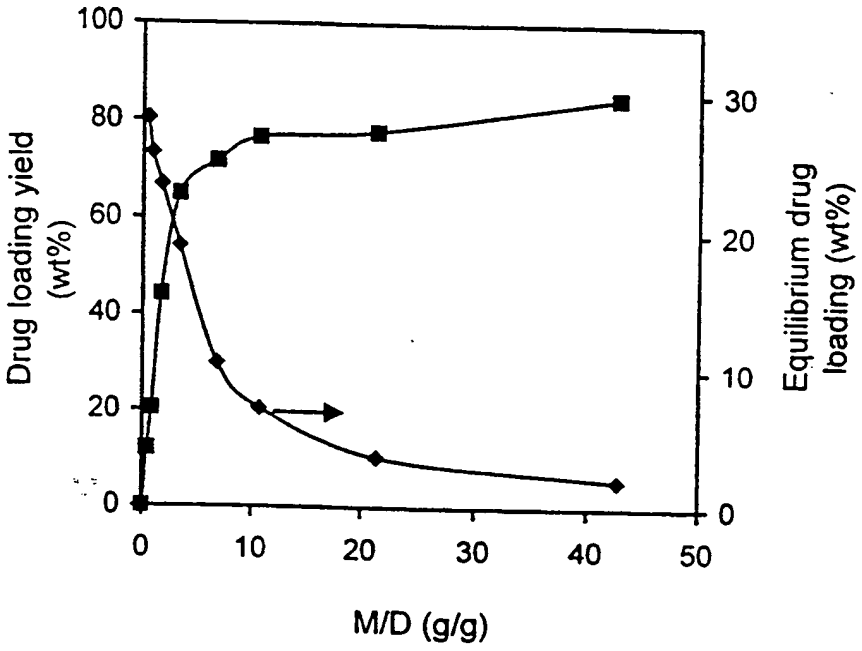

100
80
60
40
20
0
Drug loading yield (wt%)
30
20
10
0
Equilibrium drug loading (wt%)
0
10
20
30
40
50
M/D (g/g)

FIGURE 1C

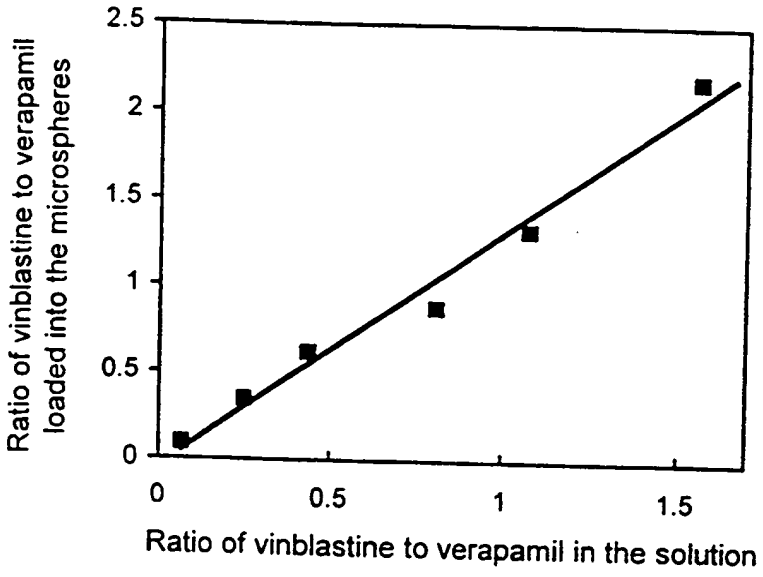

Ratio of vinblastine to verapamil loaded into the microspheres
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
Ratio of vinblastine to verapamil in the solution

FIGURE 2A

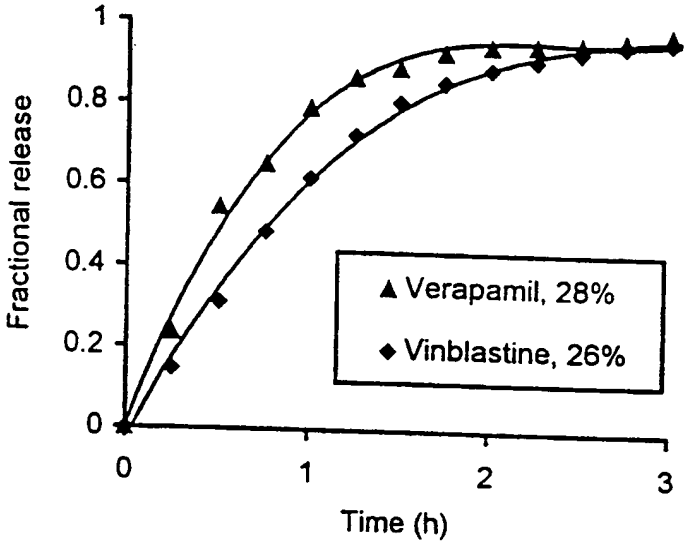

Fractional release
1
0.8
0.6
0.4
0.2
0
0
1
2
3
Time (h)
▲ Verapamil, 28%
◆ Vinblastine, 26%

FIGURE 2B

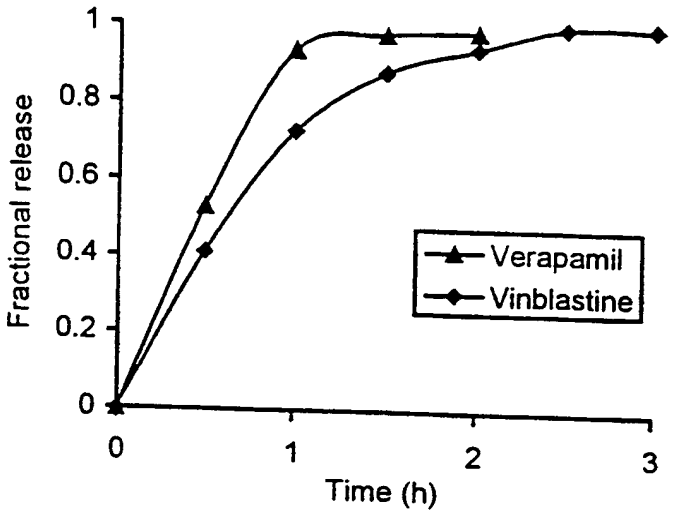

Fractional release
1
0.8
0.6
0.4
0.2
0
0
1
2
3
Time (h)
Verapamil
Vinblastine

FIGURE 3

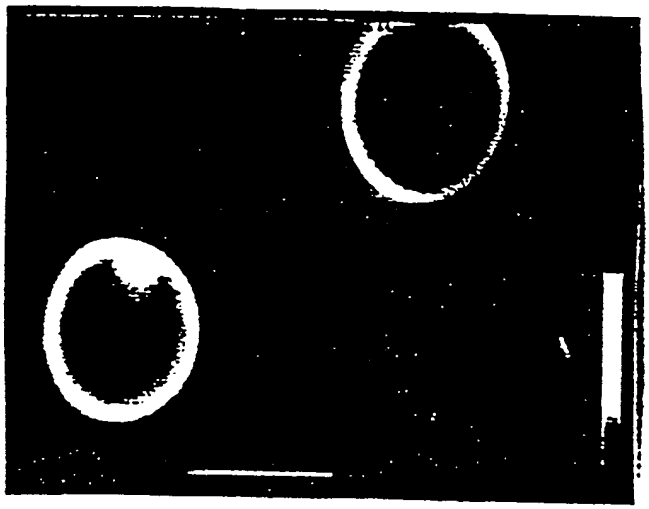


FIGURE 4

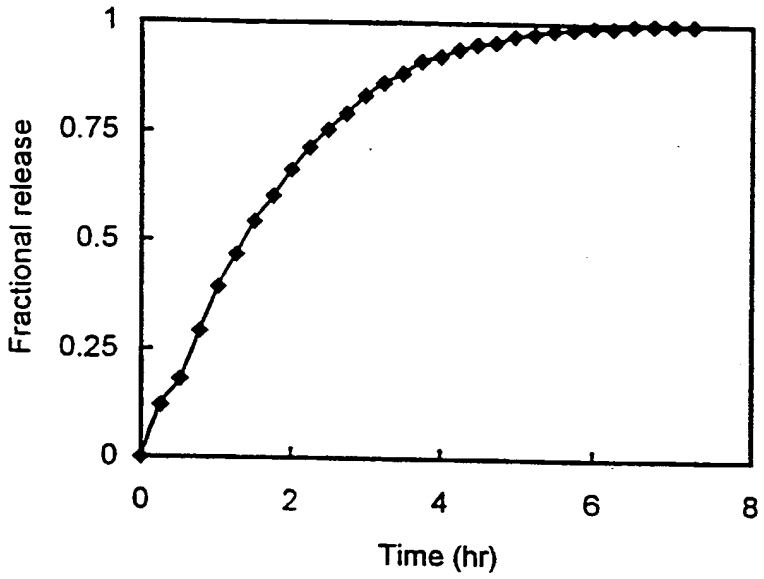


FIGURE 5

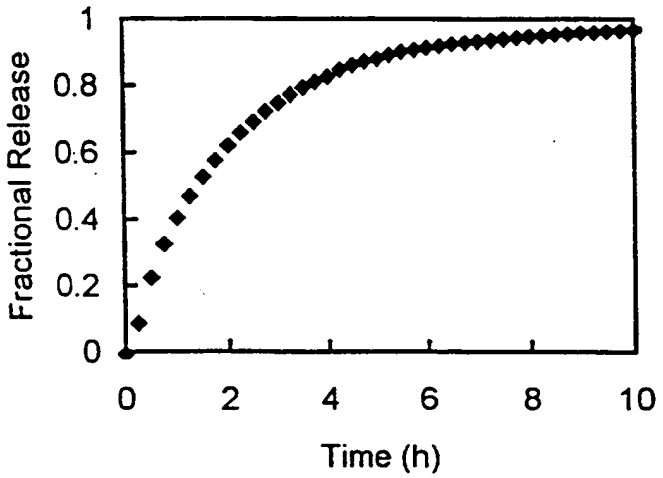


FIGURE 6

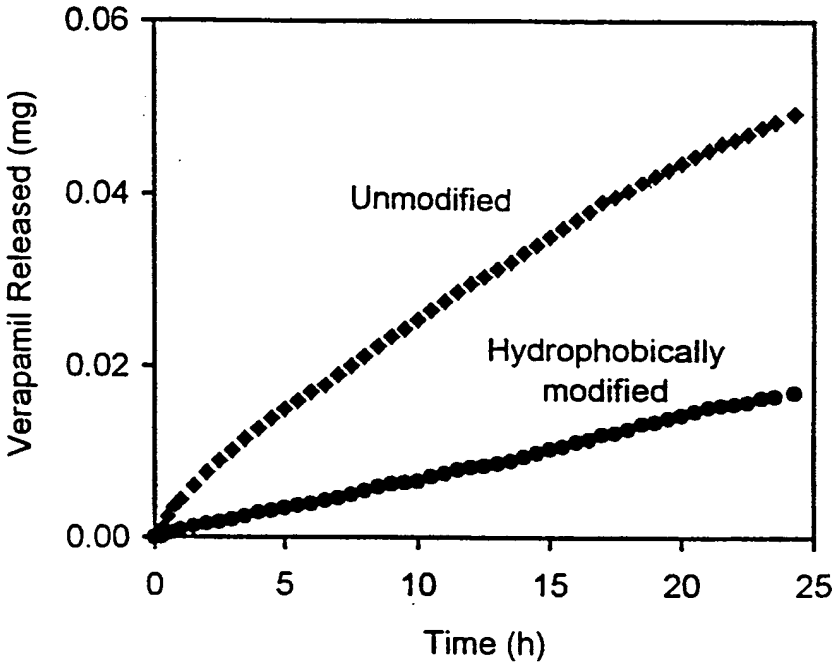

0.06
0.04
0.02
0.00
Verapamil Released (mg)
Unmodified
Hydrophobically
modified
0
5
10
15
20
25
Time (h)

FIGURE 7

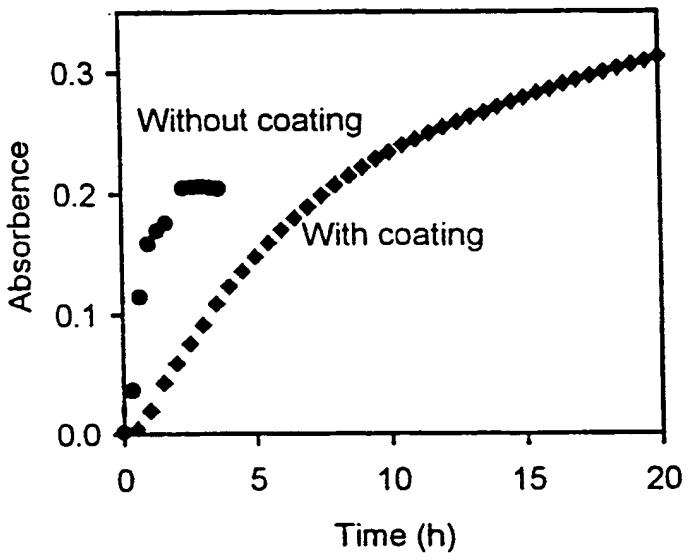

0.3
0.2
0.1
0.0
Absorbence
Without coating
With coating
0
5
10
15
20
Time (h)

FIGURE 8

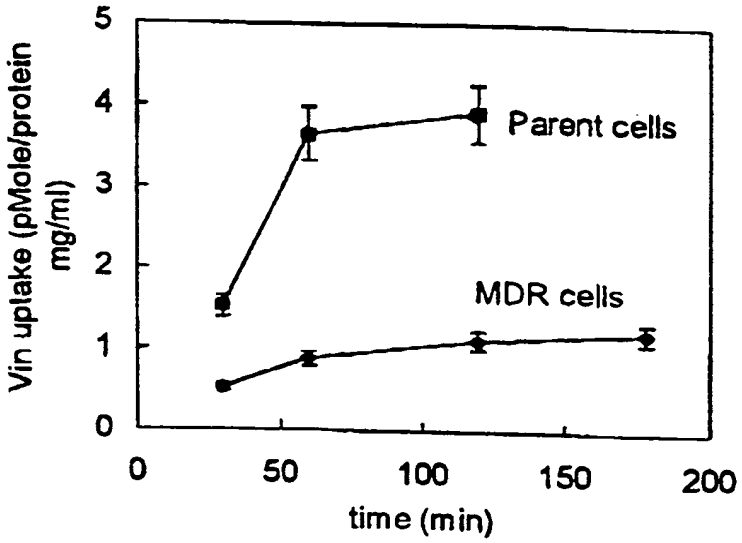

Vin uptake (pMole/protein mg/ml)
5
4
3
2
1
0
0
50
100
150
200
time (min)
Parent cells
MDR cells

FIGURE 9

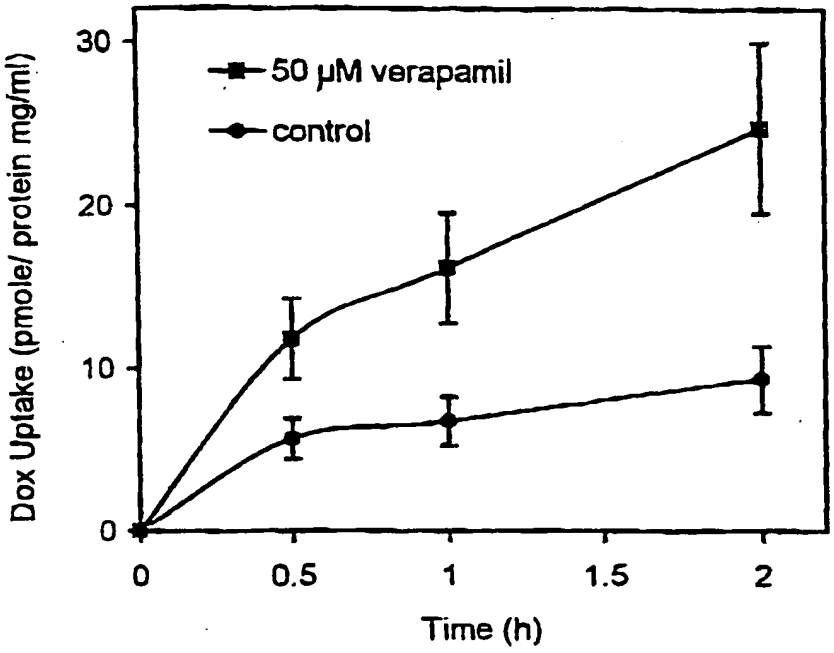

30
20
10
0
Dox Uptake (pmole/ protein mg/ml)
50 µM verapamil
control
0
0.5
1
1.5
2
Time (h)

FIGURE 10

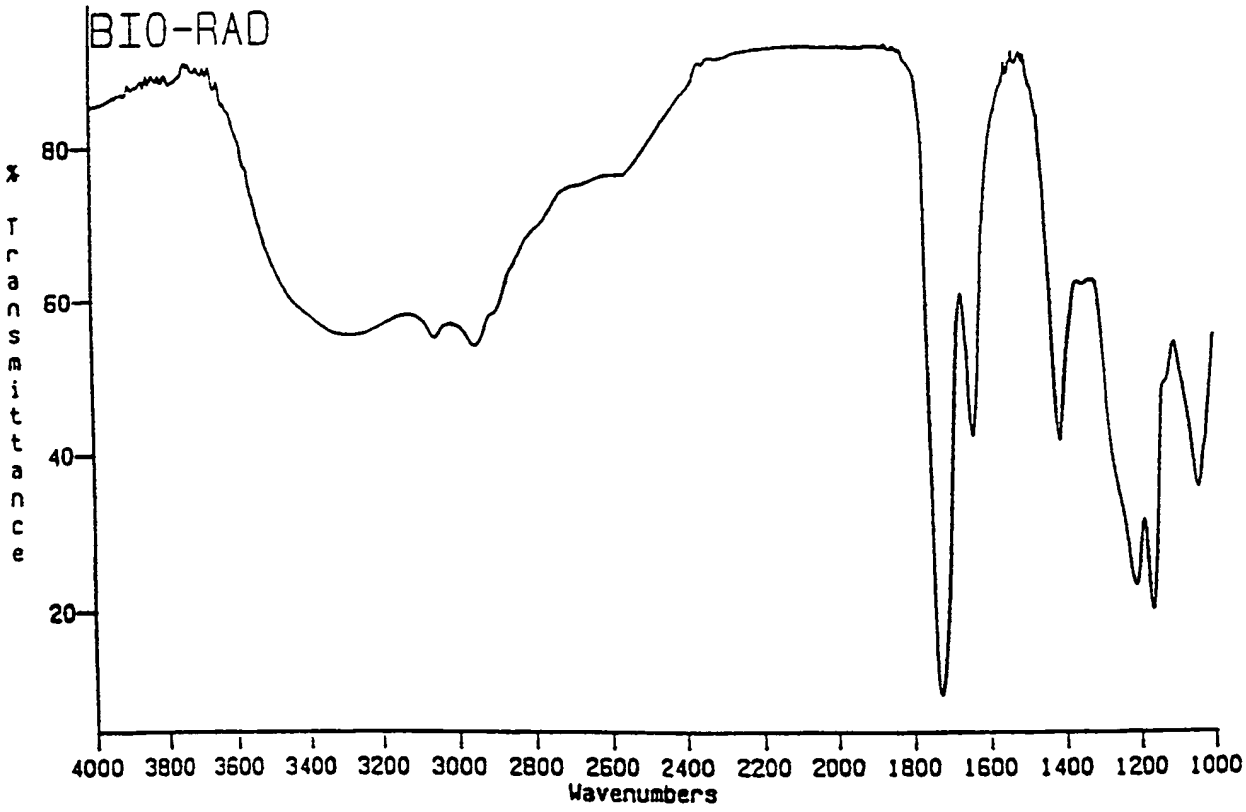

BIO-RAD
% Transmittance
80
60
40
20
4000 3800 3600 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000
Wavenumbers

FIGURE 11

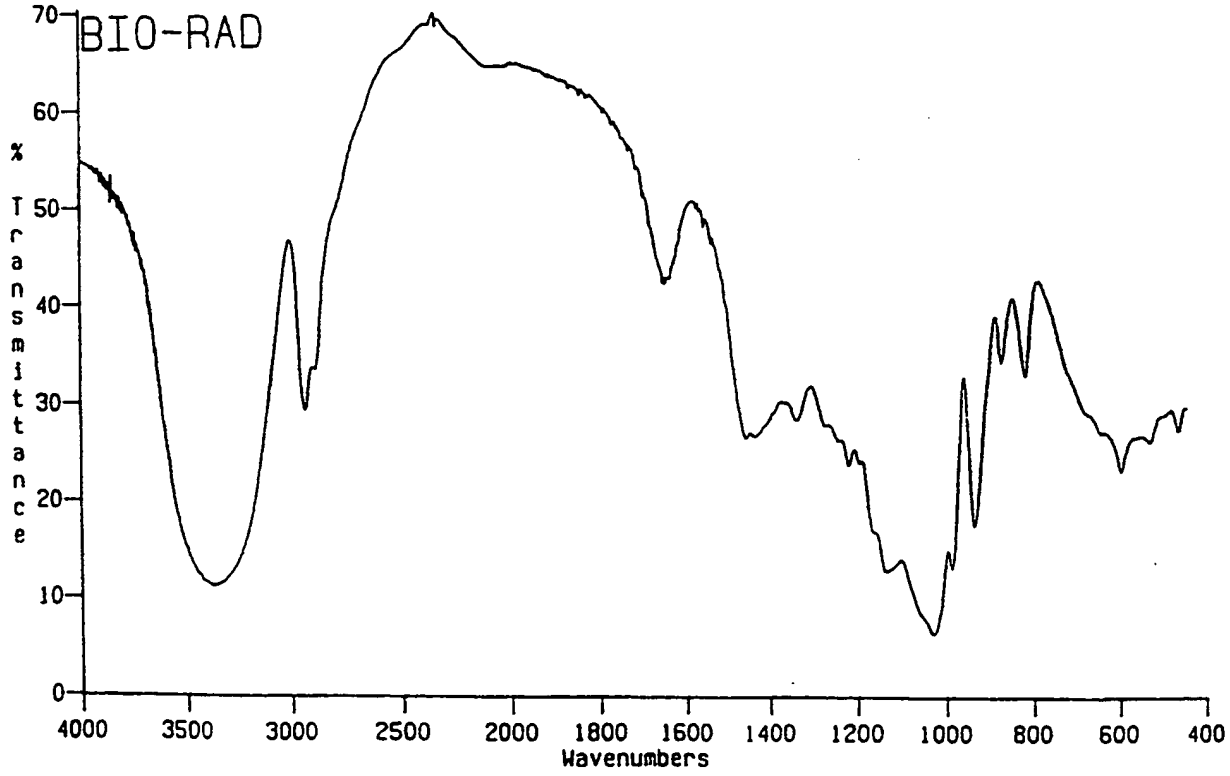
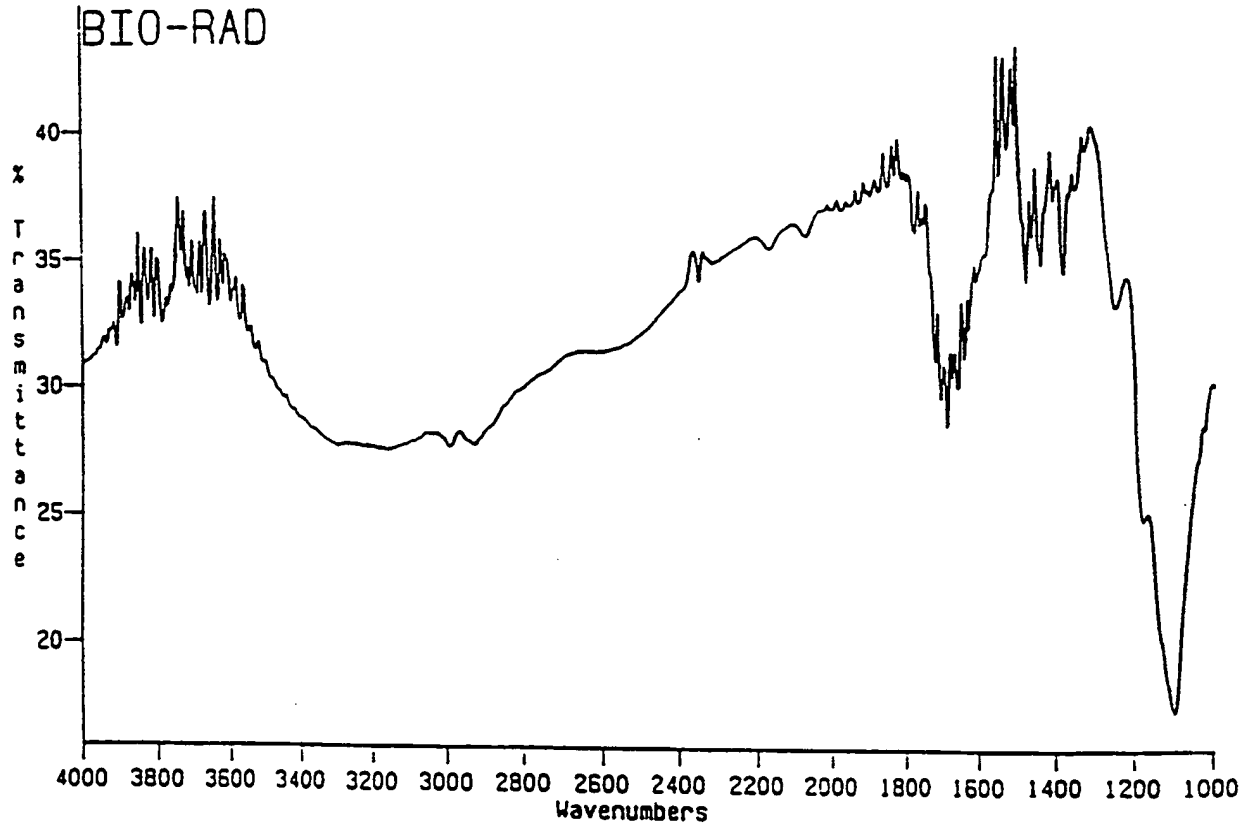

BIO-RAD
% Transmittance
70
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumbers

FIGURE 12


BIO-RAD
% Transmittance
40
35
30
25
20
4000 3800 3600 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000
Wavenumbers

INTERNATIONAL SEARCH REPORT

Interna. .al Application No
PCT/CA 98/00419

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/16 A61K47/48 A61K45/06 //A61K31/475,31:27

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CH 681 780 A (PATRINOVE;INST NAT SANTE RECH MED) 28 May 1993 see column 1, line 1-15 see column 2, line 20 - column 3, line 30 see claims 1,2,4-7,11 ---	1,15,16, 20-24, 35,36
A	EP 0 595 133 A (BEHRINGWERKE AG ;HOECHST SA LAB (FR)) 4 May 1994 see page 7, line 12-26 see examples 7,8,23,24 see claims 2,3,12,13 --- -/--	1,2,6, 15,16, 20-25, 35,36

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 September 1998	23/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	La Gaetana, R

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/CA 98/00419

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 057 304 A (KRETZSCHMAR ROLF ET AL) 15 October 1991 see the whole document	1,15,16, 20-24, 35,36
A	US 4 666 641 A (FICKAT RENE ET AL) 19 May 1987 see column 2, line 14-39 see column 5, line 44 - column 6, line 31 see claim 16	1,6, 20-22, 30,31
A	FR 2 702 160 A (BIOVECTEURS AS) 9 September 1994 see page 1, line 1-9 see page 3, line 15-18 see page 5, line 1-4 see example 14	1,2,8, 13,14, 20-22, 30,31
A	WO 94 02106 A (UNIV TEXAS) 3 February 1994 see page 1, line 10-17 see page 13, line 7-14 see page 14, line 14-21 see claims 1,2,10,13	1,6,8, 17-22
A	WO 97 09036 A (KNOTT TREVOR JOHN ;LESLIE STEWART THOMAS (GB); BROWN ADRIAN (GB);) 13 March 1997 see page 2, line 27-28 see page 3, line 17 see page 3, line 26-28 see page 4, line 19-32	1,2,8, 13, 20-22, 30,31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 98/ 00419

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 35
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 35 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-15,20,21,23,25-35
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out. specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant. this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee. this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant. this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/CA 98/00419

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/CA 98/00419

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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